PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference Case 21864WO	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/CH2004/000511	International filing date *(day/month/year)* 16.08.2004	Priority date *(day/month/year)* 14.08.2003
International Patent Classification (IPC) or national classification and IPC C12N15/53, C12N15/11, C12N9/02, C12N9/04, C12N15/63, C12N1/21, C12P17/04, C12P7/60		
Applicant DSM IP ASSETS B.V. et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 11 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☐ *sent to the applicant and to the International Bureau)* a total of sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
14.03.2005	21.11.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Devijver, K Telephone No. +31 70 340-

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/CH2004/000511

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

   Description, Pages

   1-43 as originally filed

   Sequence listings part of the description, Pages

   1-23 as originally filed

   Claims, Numbers

   1-37 as originally filed

   ☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 24-37 (in part)

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the said claims Nos. 24-37 (in part)

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has:
   - ☐ restricted the claims.
   - ☐ paid additional fees.
   - ☐ paid additional fees under protest.
   - ☒ neither restricted nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-23 (completely); 24-37 (in part) .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2-4,8,11,13-37
	No:	Claims	1,5-7,9,10,12
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-37
Industrial applicability (IA)	Yes:	Claims	1-37
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

   and / or

2. Non-written disclosures (Rule 70.9)

   see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☒ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☒ contained in the international application as filed

   ☒ filed together with the international application in computer readable form

   ☐ furnished subsequently to this Authority for the purposes of search and/or examination

   ☐ received by this Authority as an amendment on

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

1. DOCUMENTS

1.1 Reference is made to the following documents:

D1: SAITO Y ET AL: "CLONING OF GENES CODING FOR L-SORBOSE AND L-SORBOSONE DEHYDROGENASES FROM GLUCONOBACTER OXYDANS AND MICROBIAL PRODUCTION OF 2-KETO-L-GULONATE, A PRECURSOR OF L-ASCORBIC ACID, IN A RECOMBINANT G. OXYDANS STRAIN" APPLIED AND ENVIRONMENTAL MICROBIOLOGY, WASHINGTON,DC, US, vol. 63, no. 2, 1997, pages 454-460, XP000886144 ISSN: 0099-2240

D2: DATABASE EMBL [Online] 18 December 2001 (2001-12-18), "Agrobacterium tumefaciens str. C58 linear chromosome, section 35 of 187 of the complete sequence." XP002321379 retrieved from EBI accession no. EM_PRO:AE009265 Database accession no. AE009265

D3: WO 97/04101 A (FRAUNHOFER-GESELLSCHAFT ZUR FOERDERUNG DER ANGEWAND; WISSLER, JOSEF; F) 6 February 1997 (1997-02-06)

D4: WO 03/016508 A (CERESTAR HOLDING B.V; DE TROOSTEMBERGH, JEAN-CLAUDE, MARIE-PIERRE, GHI) 27 February 2003 (2003-02-27)

D5: SUGISAWA T ET AL: "ISOLATION AND CHARACTERIZATION OF A NEW VITAMIN C PRODUCING ENZYME (L-GULONO-GAMMA-LACTONE DEHYDROGENASE) OF BACTERIAL ORIGIN" BIOSCIENCE, BIOTECHNOLOGY AND BIOCHEMISTRY, XX, XX, vol. 59, no. 2, February 1995 (1995-02), pages 190-196, XP001084987 ISSN: 0916-8451

D6: WO 03/104445 A (ROCHE VITAMINS AG; HOSHINO, TATSUO; MIYAZAKI, TARO; SUGISAWA, TERUHIDE) 18 December 2003 (2003-12-18)

D7: WO 2004/029269 A (DSM IP ASSETS B.V; HOSHINO, TATSUO; MIYAZAKI, TARO; SUGISAWA, TERUHIDE) 8 April 2004 (2004-04-08)

D8: WO 03/089634 A (ROCHE VITAMINS AG; HOSHINO, TATSUO; MIYAZAKI, TARO; SUGISAWA, TERUHIDE) 30 October 2003 (2003-10-30)

D9: WO 2004/029235 A (DSM IP ASSETS B.V; HOSHINO, TATSUO;

MIYAZAKI, TARO; SUGISAWA, TERUHIDE) 8 April 2004 (2004-04-08)

D10: LEE H-W ET AL: "Screening for L-sorbose and L-sorbosone dehydrogenase producing microbes for 2-keto-L-gulonic acid production" JOURNAL OF INDUSTRIAL MICROBIOLOGY AND BIOTECHNOLOGY, BASINGSTOKE, GB, vol. 23, no. 2, August 1999 (1999-08), pages 106-111, XP002241676 ISSN: 1367-5435

Re Item IV.

The separate inventions/groups of inventions are:

1) claims 1-23 (completely); 24-37 (in part)
Isolated polynucleotide derivable from a polynucleotide encoding a polypeptide having L-sorbosone dehydrogenase activity relating to SEQ ID NO 1. Partial sequences thereof. Polypeptide encoded by such a polynucleotide relating to SEQ ID NO 2. Partial sequences thereof. Expression vector and recombinant organism comprising such polynucleotide. Process for the production of L-ascorbic acid from a substrate selected from D-sorbitol, L-sorbose and L-sorbosone using such a recombinant organism, a non-recombinant microorganism or such a polypeptide. Process for the production of L-sorbosone dehydrogenase. Process for the production of vitamin C comprising converting a substrate into vitamin C in a medium comprising resting cells of a microorganism, limited to the microorganisms as described above (microorganism comprising a polypeptide relating to SEQ ID NO 2).

2) claims 24-37 (in part)
Process for the production of vitamin C comprising converting a substrate into vitamin C in a medium comprising resting cells of a microorganism, as far as not covered by invention 1.

They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons:

Polynucleotides encoding polypeptides having L-sorbosone dehydrogenase activity and

use thereof in a process for producing L-ascorbic acid were already state of the art before the priority date of the present application. In particular, document D1 discloses (cf. abstract, page 456 and figure 5) the cloning of the gene coding for L-sorbosone dehydrogenase from Gluconobacter oxydans and its use in the preparation of L-ascorbic acid.

Processes for the production of vitamin C comprising converting a substrate into vitamin C in a medium comprising resting cells of a microorganism were also already state of the art before the priority date of the present application. In particular, document D5 discloses (cf. abstract, page 191 right-hand column paragraph 2)b) and table II) Gluconobacter oxydans DSM 4025 producing 13.9 g/l L-ascorbate from L-gulono-gamma-lactone; cells are allowed to reach the resting state and are thereupon transferred to a separate vessel for reaction.

In the light of the above mentioned prior art, the problems and corresponding solutions of the present application can be summarized as follows:

problem 1: providing further polynucleotides encoding polypeptides having L-sorbosone dehydrogenase activity which can be used in a process for producing L-ascorbic acid;

solution 1: polynucleotides relating to SEQ ID NO 1 encoding polypeptides relating to SEQ ID NO 2 (and their uses);

problem 2: providing further processes for the production of vitamin C comprising converting a substrate into vitamin C in a medium comprising resting cells of a microorganism;

solution 2: process for the production of vitamin C comprising converting a substrate into vitamin C in a medium comprising resting cells of a microorganism (as far as not covered by invention 1).

The ISA considers that, due to the fact that polynucleotides encoding polypeptides having L-sorbosone dehydrogenase activity and use thereof in a process for producing L-ascorbic acid and processes for the production of vitamin C comprising converting a substrate into vitamin C in a medium comprising resting cells of a microorganism were known (cf. D1 and

D5), due to the essential differences between the aforementioned problems and corresponding solutions, and due to the fact that no other technical feature can be distinguished which in the light of the prior art could be regarded as special technical feature, there is no single inventive concept underlying the plurality of claimed inventions, and an objection for non-unity of invention has to be raised under PCT Rule 13.1. Consequently, there is a lack of unity and the different inventions, not belonging to a common inventive concept, are formulated as the different subjects on the communication pursuant to Art. 17(3)(a) PCT.

The application relates to a plurality of inventions, or groups of inventions, in the sense of Rule 13.1 PCT. They have been divided as defined above. If the applicant pays additional fees for one (or more) not yet searched group(s) of invention(s), then the further search(es) may reveal further prior art that gives evidence of a further lack of unity 'a posteriori' within one (or more) of the not yet searched group(s). In such a case only the first invention in this (each of these) group(s) of inventions, which is considered to lack unity of invention, will be the subject of a search. No further invitation to pay further additional fees will be issued. This is because Article 17(3)(a) PCT stipulates that the ISA shall establish the International Search Report on those parts of the international application which relate to the invention first mentioned in the claims ('main invention') and for those parts which relate to inventions in respect of which the additional fees were paid. Neither the PCT nor the PCT guidelines provide a legal basis for further invitations to pay further additional search fees (W17/00, point 11 and W1/97, points 11-16).

Re Item V.

2. NOVELTY (Art. 33(2) PCT)

2.1 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1, 5-7, 9, 10 and 12 is not new in the sense of Article 33(2) PCT.

2.2 Document D2 discloses (cf. the whole document) an isolated polynucleotide comprising a partial nucleotide sequence of at least 20 consecutive nucleotides of

SEQ ID NO 1 (residues 2323-2342) and SEQ ID NO 26 (residues 2323-2342). The expression "derivable from a polynucleotide encoding a polypeptide having L-sorbosone dehydrogenase activity" of claim 1 does not have any limiting effect on the scope of the claim, i.e. the claim is directed to the product per se. The same comment applies to the term "recombinant" of claim 12. Consequently, D2 anticipates the subject-matter of claims 1, 5-7 and 12.

2.3 Document D3 discloses (cf. SEQ ID NOs 7, 12 and 20) polypeptides comprising a partial amino acid sequence of at least 25 consecutive amino acids selected from the group consisting of SEQ ID NOs 2, 12, 18 and 27. Consequently, D3 anticipates the subject-matter of claims 9 and 10.

3. INVENTIVE STEP (Art. 33(3) PCT)

3.1 The present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1-37 does not involve an inventive step in the sense of Article 33(3) PCT.

3.2 Document D1 is considered to represent the most relevant state of the art and discloses (cf. abstract, page 456 and figure 5) the cloning of the gene coding for L-sorbosone dehydrogenase from Gluconobacter oxydans and its use in the preparation of L-ascorbic acid. The subject-matter of the present application differs in that a further L-sorbosone dehydrogenase polypeptide (relating to SEQ ID NO 2) and corresponding polynucleotide (relating to SEQ ID NO 1) are provided.

3.3 The problem to be solved by the present application may therefore be regarded as providing a further L-sorbosone dehydrogenase polypeptide/polynucleotide. The proposed solution is the L-sorbosone dehydrogenase polypeptide, relating to SEQ ID NO 2, and the corresponding polynucleotide, relating to SEQ ID NO 1.

3.4 This solution cannot however be considered as involving an inventive step for the following reasons. The provision of this molecule is regarded as obvious, because in

view of the prior art (cf. D10), the skilled person has an incentive to isolate further L-sorbosone dehydrogenases due to their importance in 2-keto-L-gulonic acid (2KGA) and vitamin C production. Moreover, the provision of such molecules is obvious, as they are identified without any difficulties as already demonstrated in the prior art (cf. D10); this is also apparent from the description of the present application. Consequently, the subject-matter of the present application does not involve an inventive step. The routine provision of further sequences having the same general function as the known prior art sequences is not inventive. The structural non-obviousness per se is not sufficient to accept an inventive step, because a specific DNA sequence must be composed of a succession of defined deoxyribonucleotides, whichever this is and, therefore, it cannot be considered inventive for this sole reason. Inventive step can only be acknowledged if the specific succession of deoxyribonucleotides imparts some unexpected useful properties and/or technical effect to the molecule.

3.5 The fact that vitamin C is produced using the L-sorbosone dehydrogenase of the present application is not an unexpected property and/or technical effect, because vitamin C is always formed during such a reaction (cf. D4 examples 1-7 and D1 figure 5).

3.6 The other claims do not contain any features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step (Article 33(3) PCT).

4. FURTHER REMARKS

4.1 It appears that presently claimed priority is not valid for subject-matter relating to SEQ ID NOs 23-27, 30 and 31. Consequently, documents D6-D9 may be taken into account for the assessment of novelty and/or inventive step concerning said subject-matter.